

OL-055 A Multiplex PCR-Based Reverse Line Blot Hybridization (mPCR/RLB) strategy for subtyping the staphylococcal cassette chromosome mec (SCCmec) type IV in methicillin-resistant *Staphylococcus aureus*

Ying Liu^{*1}, Fanrong Kong², Meng Xiao³, Qinning Wang², Matthew O'Sullivan⁴, Gwendolyn L. Gilbert². ¹Department of Dermatology, Beijing Children's Hospital, Beijing, PR China; ²Centre for Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research (ICPMR), Westmead, New South Wales, Australia; ³Life Science College, Peking University, Beijing, PR China; ⁴Western Clinical School, University of Sydney, Sydney, Australia

Objectives: To develop and validate a multiplex PCR-based reverse line blot hybridization (mPCR/RLB) strategy for subtyping staphylococcal cassette chromosome mec (SCCmec) type IV methicillin-resistant *Staphylococcus aureus* (MRSA) strains.

Methods: Sixty primer pairs and 63 probes were designed on the basis of the available sequences of each open-reading frame (ORF) at GenBank, for the previously described SCCmec IV subtypes. Probes were compared *in silico* to 18 whole genome sequences complete genome and partial SCCmec gene of 6 non-MRSA strains, including methicillin susceptible *S. aureus* and methicillin resistant coagulase negative staphylococci.

Results: The mPCR/RLB assay classified a set of 93 MRSA strains which possessed SCCmec type IV into 68 subtypes, including 46 subtypes for 52 well-characterized reference strains and 22 subtypes for 41 clinical strains. The discriminatory power (Simpson indices of diversity of 0.987) is higher than other genotyping methods to date. It can rapidly and simultaneously detect 43 samples with a turn-around time (including culturing of isolates, DNA extraction, mPCR setup and running, and RLB hybridization) of about two working days. This comparative analysis the potential sensitivity of probes demonstrated that each of the 63 probes found homologous match with at least one GenBank MRSA SCCmec type IV sequence.

Conclusion: The application of mPCR/RLB hybridization assay to MRSA SCCmec IV subtyping can improve the specificity, discriminatory power and throughput of the typing procedure. Moreover it is also a good tool for near-real time infection control surveillance and MRSA transmission tracking.

OL-056 Biochemical and immunological parameters of amniotic fluid from pregnant women with herpes virus infection and congenital defects of fetuses

Galyna Gaidai^{*1}, Larysa Bondarenko². ¹O.O. Bogomoletz National Medical University, Kiev, Ukraine; ²SI Institute of Pharmacology and Toxicology Academy of Medical Science of Ukraine, Kiev, Ukraine

Early diagnosis and appropriate treatment of herpes virus infections of pregnant women allows to minimize levels of inborn herpes virus infections and fetal congenital defects.

Results of this pathology prenatal diagnostics often have ambiguous and contradictory character. High levels of lethality and fetal congenital defects incidence demand creation a complex of diagnostic parameters for early stages of herpes virus infection determination and fetal health status monitoring. Our aim was to create complex of biochemical and immunological parameters for amniotic fluid investigation with the highest prognostic value concerning inborn herpes virus infection and fetal congenital defects incidence.

Materials and methods: Amniotic fluid was obtained via trans-abdominal amniocentesis from 24 pregnant women (18-35 years old) with herpes virus infection (according virological analysis) and fetal congenital defects (according to ultrasonic markers). Antibodies IgM and IgG were determined by immunoenzymatic

analysis. Biochemical parameters were investigated on biochemical analyzer "Cobas-Mira" (Austria).

Result: In first group (10 women, 16-20 weeks gestation) there were 1 positive result as to herpes virus IgG-antibodies, while in second group (14 women, 21-24 weeks gestation) - 8. Changes in biochemical parameters were statistically significant even at 16 weeks of gestation. The highest changes were demonstrated for levels of bilirubin, total protein, AST (increasing), glucose and ALT (decreasing in comparison with norm).

Conclusions: Statistical analysis demonstrated that only complex of biochemical, immunological, virological and ultrasonic investigations could have the highest prognostic value as to inborn herpes virus infection and fetal congenital defects.

OL-057 The distribution and quantification of cytomegalovirus glycoprotein gB genotypes in serum

Xuan Zhang^{*}, Jun Fan, Meifang Yang, Hainv Gao, Xiaoming Chen, Hong Zhao, Jianhua Hu, Weihang Ma. State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China

Background: Based on the sequence variation in the N-terminus of the UL55 gene, which encodes glycoprotein B (gB), human cytomegalovirus (CMV) can be classified into four gBn genotypes. This study determined the distribution and quantification of CMV gBn genotypes in a Chinese population of HSCT recipients and examined the correlations among the gBn genotype, pp65 antigen, CMV gBn DNA, and clinical outcome.

Methods: The distribution of CMV gBn genotypes and the level of gBn DNA were detected by real-time PCR. CMV-positive pp65 cells were identified by immunohistochemical staining.

Result: The distribution of gB genotypes was as follows: gBn1, 60% of patients; gBn2, 13.3%; mixed gBn1 and gBn3 infection, 26.7%; and gBn4 and other mixed infections, 0%. The detected level of CMV gB DNA correlated well with the number of CMV-positive pp65 cells ($r=0.514$).

Conclusion: The distribution of CMV gBn genotypes in a Chinese HSCT recipients were determined. And gBn1 genotype is predominant. A statistical relationship between the different gB types and the clinical outcome could be not determined, owing to the small sample size in this study.

OL-058 Methylation status of hepatic IGF-II promoter 3 in HBV-related chronic liver diseases

Jing Qian^{*}, Dengfu Yao, Yucheng Shen, Shanshan Li, Yinzu Bian. Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, Nantong, China

Objective: To investigate the promoter methylation status of IGF-II in hepatoma model and human HCC tissues for exploring its mechanism at the occurrence of HCC.

Methods: Hepatoma models were induced with 2-FAA on male SD rats. Morphological changes of livers were observed by H&E staining. The CpG island methylation status of rat and human IGF-II gene P was observed by MSP.

Results: IGF-II was overexpressed in hepatocytes from granule-like degeneration to atypical hyperplasia and HCC development. The levels of serum IGF-II expressions in HCC were significantly higher than any of other groups ($P<0.01$). The incidence of P2 methylation was 100% in normal, 83.3% in degeneration, 11.1% in precancerous, and 0% in HCC group, respectively. The rates of IGF-II P3 methylation in was 0% in human HCC, 47.5% in their surrounding, and 100% in normal tissues, respectively, with significant differences among them ($P=0.000$). No significant significance was found between the methylation rates of IGF-II P3 and clinical parameters. However, the methylation